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**(54) Title:** STABLE STERILE FILTERABLE LIPOSOMAL ENCAPSULATED TAXANE AND OTHER ANTINEOPLASTIC DRUGS

**(57) Abstract:** The invention provides a formulation of one or more antineoplastic drugs encapsulated in liposomes including at least a lipid fraction in addition to the antineoplastic drug, wherein the composition is stable in an aqueous solution at room temperature.

# STABLE STERILE FILTERABLE LIPOSOMAL ENCAPSULATED TAXANE AND OTHER ANTINEOPLASTIC DRUGS

## CROSS-REFERENCE TO RELATED APPLICATIONS

**[0001]** This application claims priority to co-pending United States Provisional Patent Application 60/444,958, filed on February 3, 2003, the entirety of which is incorporated herein by reference thereto.

## TECHNICAL FIELD OF THE INVENTION

**[0002]** The present invention relates to liposomal encapsulated taxane and other antineoplastic drugs.

## BACKGROUND OF THE INVENTION

**[0003]** The use of taxanes, such as paclitaxel, as anti-tumor agents for patients suffering from diseases, such as ovarian and breast cancer, is known. In addition, paclitaxel has been shown to be clinically potent as a synergistic agent when used in conjunction with radiation treatment. Paclitaxel has a unique mechanism of action and a broad spectrum of anticancer activity because paclitaxel shows stabilization of microtubules rather than disassembly of microtubules.

**[0004]** However, paclitaxel has extremely low solubility in water, which makes it difficult to provide a suitable dosage form. Currently, paclitaxel is prepared and administered in a vehicle containing 6 mg/mL paclitaxel, 527 mg/mL of purified cremophor EL (a polyethoxylated castor oil), and 49.7% dehydrated alcohol, USP. This solution is diluted 1:10 in saline before being administered to humans. The stability of paclitaxel once diluted in saline solution is quite low. The drug degrades within 24 hours, and it has been shown that taxol is incompatible with common PVC intravenous bag and infusion sets, thus, handling of dosage for the patients becomes very difficult. Since the drug precipitates from dilution, an on-line filter is utilized for the infusion of the drug to the patients. The decreased solubility and presence of Cremophor EL in the formulations presents risks to patients, such as anaphylactoid reactions and cardiotoxicity. The long-term use of taxol also can contribute to the development of multidrug resistence in cancer cells, which only complicates the etiology of the very disease for which taxol treatment is sought.

**[0005]** Attempts have been made to improve upon the currently-available formulations of taxol. To this end, U.S. patent 5,648,090 (Rahman et al.) and U.S. Patent 5,424,073 (Rahman et al.) provide a liposomal encapsulated paclitaxel for a method for treating cancer in mammals using such a liposomal-encapsulated paclitaxel,

or antineoplastic derivative thereof. The '090 and '073 patents disclose a method of modulating multidrug resistance in cancer cells in a mammalian host by administering to the host a pharmaceutical composition of a therapeutically effective number of liposomes, which include a liposome-forming material, cardiolipin, and an agent such as paclitaxel, or an antineoplastic derivative of paclitaxel, or a mixture thereof, and a pharmaceutically acceptable excipient. However, there remains a need for a liposomal formulation of taxanes that remain stable for a prolonged period of time.

## SUMMARY OF THE INVENTION

**[0006]** The present invention provides a formulation of antineoplastic drugs, such as taxanes, derivatives thereof, and related compounds, at clinically relevant concentration that exhibits improved stability and reduced toxicity. The inventive formulation preferably contains one or more stabilizing agents, antioxidants, and lyoprotectants. In many preparations, the inventive formulation is stable for many days at room temperature, even after post-reconstitution and dilution in injectable fluids. The inventive formulation can increase therapeutic efficacy and minimize multidrug resistance over that observed with present taxane formulations. These and other advantages of the present invention, as well as additional inventive features, will be apparent from the description of the invention provided herein and from the accompanying figures.

## DESCRIPTION OF THE FIGURES

**[0007]** Figure 1 graphically presents the results of a study of the effect of liposome entrapped paclitaxel (LEP) and Taxol on growth of ectopic human lung tumor (A549) in SCID mice.

**[0008]** Figure 2 graphically presents the results of a study of the effect of liposome entrapped paclitaxel (LEP) and Taxol on body weight of human lung tumor bearing SCID mice.

## DETAILED DESCRIPTION OF THE INVENTION

**[0009]** The present invention is predicated, at least in part, on providing a formulation of one or more antineoplastic drugs encapsulated in liposomes including at least a lipid fraction in addition to the antineoplastic drug, wherein the composition is stable in an aqueous solution at room temperature, typically stable for at least 3 days. Preferably, the formulation is free or substantially free of antineoplastic drug crystals or precipitate, and most preferably, there are no antineoplastic drug crystal and precipitate forms in the formulation.

[0010] Any suitable antineoplastic drug can be used in the context of the invention. Preferred compounds for use in the inventive formulation include taxanes or derivatives, such as docetaxel, paclitaxel and related compounds (e.g., epothilones A and B, epothilone derivatives, etc.). Preferably, the compound is paclitaxel. A suitable derivative of paclitaxel is taxane. Other suitable compounds are 7-epipaclitaxel, t-acetyl paclitaxel, 10-desacetyl-paclitaxel, 10-desacetyl-7-epipaclitaxel, 7-xylosylpaclitaxel, 10-desacetyl-7-glutarylpaclitaxel, 7-N,N-dimethylglycylpaclitaxel, 7-L-alanylpaclitaxel, taxotere, and mixtures thereof.

[0011] As the invention provides a liposomal formulation of antineoplastic drug, the formulation also includes one or more compounds able to form liposomes. Thus, for example, the formulation can include cholesterol or derivatives thereof, lipids or phospholipids, and other similar compounds. Preferably, the lipid fraction of the inventive liposomal formulation includes one or more cardiolipins, such as synthetic or naturally occurring cardiolipins or cardiolipin analogues.

[0012] Typically, the lipid fraction comprises at least about 3.5% (w/v) and more preferably at least about 4.0% (w/v) of the inventive composition, such as at least about 5.0% or even at least about 5.5% (w/v) of the composition. Generally, it is not expected that the lipid fraction will exceed about 8.5% (w/v), and more preferably, the maximal lipid fraction will be about 8.0% (w/v) of the composition, such as a top lipid concentration of about 7.0% (w/v) or even a maximal lipid concentration of about 6.0% (w/v). Preferably, the liposomes of the inventive formulation will have between about 4.0% (w/v) and about 8.0% (w/v) lipid, such as between about 5.0% (w/v) and about 6.0% (w/v) lipid.

[0013] Moreover, the ratio of lipid to antineoplastic drug used in the inventive formulation typically is at least about 5:1 by molar ratio, and more preferably at least about 10:1 by molar ratio, at least where the antineoplastic drug is a taxane (or related compound or derivative thereof). Typically, the ratio of lipid to antineoplastic drug used in the inventive formulations is at least about 20:1 by molar ratio, such as at least about 30:1 or even 40:1 by ratio. However, generally, the ratio of lipid to antineoplastic drug used in the inventive formulation generally does not exceed about 75:1 by molar ratio, and is more typically at most about 70:1 by molar ratio. Typically, the ratio of lipid to antineoplastic drug, especially a taxane (or related compound or derivative) used in the inventive formulations is at most about 60:1 by molar ratio, such as at most about 50:1 or even at most about 40:1 by molar ratio. Preferably, the ratio of lipid to drug in the inventive formulation is between about 10:1 and about 70:1 by molar ratio, such as between about 25:1 and about 55:1 by molar ratio.

[0014] Desirably, a majority of the antineoplastic drug in the formulation is entrapped in the liposomes. More preferably, at least about two-thirds (such as at least about 75%) of the antineoplastic drug in the formulation is entrapped in the liposomes, and it is even more preferable for at least about 85% (or even more than about 90%) of the antineoplastic drug to be entrapped in the liposomes in the inventive formulation.

[0015] Preferably the lipid fraction of the formulation includes one or more lipids selected from the group consisting of 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), tetramyristoyl cardiolipin (CL), and cholesterol (CH), as these constituents lend stability to the formulation and can function as stabilizing agents. Other saturated phospholipids such as dimyristolphosphatidylcholine (DMPC), dipalmitoylphosphatidylcholine (DPPC) and distearoylphasphatidylcholine (DSPC) and unsaturated phospholipids such as hydrogenated purified soy bean phosphatidylcholine, hydrogenated purified egg yolk phosphatidylcholine, dilinoleoylphosphatidylcholine (DLPC), phasphatidylcholine (DOPC), palmitoyloleoyl dioleoylphosphatidylcholine (POPC) and sphingomyelin may be used. Suitable negatively charged lipids such as dioleoylphsophatidylglycerol (DOPG), dioleoyl phosphatidylserine (DOPS), dimyristolphosphatidylglycerol (DMPG) also can be used.

[0016] Most preferred constituents for inclusion in the lipid fraction of the inventive composition include 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), tetramyristoyl cardiolipin (CL), and cholesterol (CH). It is desirable for two or more of these lipid constituents to be present in the lipid fraction, and most preferably all three of these lipid constituents (DOPC, CL, and CH) are present. Indeed, the DOPC preferably comprises between about 85% and about 95% of the lipid components, and even more preferably between about 90% and about 92% of the lipid components. Suitable formulations include ratios of DOPC:CH:CL between about 92:0:8 to about 90:5:5, although the amounts of CH and CL do not have to be equal. In this respect, the concentration of CH typically is between about 0.3 mg/ml and about 3.5 mg/ml, such as between about 0.5 mg/ml and about 3.0 mg/ml, or even between about 1 mg/ml and about 2 mg/ml; whereas the amount of CL typically is between about 1 mg/ml and about 10 mg/ml (more typically between about 2 mg/ml and about 8 mg/ml), such as about 5 mg/ml. However, the amount of DOPC in the lipid fraction of the inventive formulation generally exceeds that of CH or CL, and typically the amount of DOPC in the inventive formulation exceeds the amount of both CH and CL combined. In this respect, the amount of DOPC in the lipid fraction of the inventive formulation typically is about 60 mg/ml, and is generally at least

about 40 mg/ml, such as at least about 45 mg/ml or even at least about 50 mg/ml. Typically, the DOPC represents no greater than about 75 mg/ml, such as at most about 65 mg/ml or 70 mg/ml. An ideal range of DOPC is between about 44 mg/ml and about 74 mg/ml.

**[0017]** To further assist in enhancing the stability, the inventive formulation can typically include one or more antioxidants, in addition to the active drug (i.e., taxane or related compounds) and the lipid fraction. While any commonly-used lipid soluble antioxidants can be employed, in some embodiments, antioxidants such as butylated hydroxyanisole, butylated hydroxytoulene and propyl gallate may be used. D-alpha tocopheryl acid succinate is preferred because of its stability-enhancing properties. Where present, the antioxidant typically is included in the formulation in concentrations between about 0.1 and about 0.6 mg/ml, such as between about 0.2 mg/ml and between about 0.5 mg/ml, or even between about 0.3 mg/ml and about 0.4 mg/ml.

**[0018]** The liposomes can be formulated by any suitable method. Preferred methods include thin film hydration, solvent injection, freeze-thawing and dehydration-rehydration, removal of surfactant, reverse phase evaporation and ethanol injection. For example, the antineoplastic drug and lipid fraction can be dissolved in a suitable solvent, such as methylene chloride, ethanol, methyl acetate, ethyl formate and the like. Where antioxidants are employed, they also can be dissolved in the solvent with the lipid fraction. For the purpose of formulating the inventive composition, any suitable amount of solvent can be employed, which typically is between about 1000 and 1100 mg/ml where methylene chloride is used as the solvent. Typically, however, the method will require at least about 500 mg/ml methylene chloride, such as least about 750 mg/ml mg/ml methylene chloride, and as much as about 2000 mg/ml methylene chloride, such as up to about 1500 mg/ml methylene chloride can be used to dissolve the lipid fraction.

**[0019]** After the antineoplastic drug, lipid fraction, and antioxidant are dissolved in the solvent, the solution then is dried. Any suitable drying methodology and apparatus can be employed, a preferred drying step comprising a rotary evaporator under reduced pressure, followed by further drying in a dessicator.

**[0020]** Following drying, the lipid (and antioxidant) residue can be hydrated in an aqueous system, such as water, which can be a solution or suspension. Preferably, the solution contains one or more lyoprotectants to aid in enhancing the stability of the formulation during subsequent lyophilization and while present in lyophilized form. Any suitable lyoprotectant, such as sugars and mannitol can be employed, although such compounds typically are sugars. While sucrose is a most preferred

lyoprotetant, other suitable lyoprotectants include, for example, trehalose, maltose, lactose, glucose, dextran, aminoglycosides and streptomycin and combinations of these can suitably be employed, as desired. Typically, the lyoprotectant represents less than about 50% (w/v) of the formulation, if it is employed. More typically, the lyoprotectant represents at most about 40% (w/v) of the formulation, such as at most about 3%0 (w/v) of the formulation. Where the lyoprotectant represents as little as about 1% (w/v), it can enhance the stability of the inventive formulations; however, more typically, the lyoprotectant represents at least about 5% (w/v) of the formulation, such as at least about 10% (w/v) or at least about 20% (w/v) of the formulation. The hydration solution also can contain a tonicity adjuster, which preferably is sodium chloride (NaCl), but can be another suitable salt or disaccharide. The hydration can be accomplished in any suitable volume of hydration solution.

**[0021]** Re-hydration in the hydration solution results in the formation of multilamellar vesicles (MLV). In certain methods, suitable preparations can be mixtures of multilamellar vesicles and unilamellar vesicles. Once the solution is added, liposomes can be formed by mixing, for example, as by vortexing or by using any suitable mixing devise. Where smaller vesicles are desirable, the solution can be sonicated. If desired, the size of these MLV can be manipulated, for example, by extrusion through a sieve, which is typically formed of polycarbonate fibers or by homogenization using high pressure homogenizer. Thus, the size of the MLV in the composition can be controlled, using a sieve of a pre-selected size (e.g., via extrusion through a sieve of a desired size, such as 0.8μm, 0.4μm, 0.2μm, 0.1μm, etc.). In the present invention, a sizing treatment is preferably applied to make the particle size of the drug-encapsulating liposomes more uniform. Prior to lyophilization, the formulations of the present invention can be sterile filtered through a 0.22 micron filter. The average particle size of the formulation is about 50 to 200 nm, preferably 100-180 nm, more preferably 120-160 nm.

**[0022]** Following re-hydration and (if desired) extrusion to achieve a defined particle size, the composition preferably is lyophilized using any suitable device or method. A preferred device is a benchtop and any suitable size of lyophilizer (e.g., such as is manufactured by VerTis). The SUV preparation can be maintained in lyophilized form (e.g., in cold storage at about –5-8°C) for an extended period of time, such as for at least about several months or years. Preferably the lyophilized formulation is stable for at least 9 months.

**[0023]** For use, the lyophilized SUV liposomal formulation can be reconstituted with a suitable volume of reconstitution solution, which preferably is a polar solvent, and most preferably an aqueous system, which can be de-ionized water or a suitable

aqueous saline solution. Any suitable volume of reconstitution solution can be employed, such as between about 1 ml and about 50 ml, more typically between about 3 ml and about 25 ml. For use, the liposomal formulation can be diluted as desired, such as in a suitable physiologically-compatible buffer or saline solution. To assist in reconstitution, the preparation can be mixed gently or vigorously (e.g., vortexed) as desired, or even sonicated.

**[0024]** However produced, the resultant liposomal-encapsulated taxane are stable for at least about one day, and more typically for at least 3 or 4 days following re-constitution. Indeed, formulations of the present invention can remain stable at room temperature for at least about 4 days, such as a week or two weeks, or even longer times. As indicated below in the Example sections, formulations of the present invention can remain stable under such conditions for at least about 17 days. Stability can be monitored, for example, by assessing the particle size of the liposomes over time (stability in this context can be indicated by a change of mean particle size of less than about 20% over one day and more preferably a change of particle size of less than about 5% in one day, or a change of less than about 10% or even 5% over a 3- or 4-day timecourse) using particle size analyzer, such as Nicomp Submicron Particle Sizer. Alternatively, stability can be assessed by assessing (e.g., using a light microscope) for the presence of crystalline taxane. The absence of more than trace amounts of such crystals is indicative of a stable preparation, and desirably, the inventive formulation will remain substantially, or even relatively completely, free of taxane crystals after three or four days of reconstitution or dilution in saline, even at room temperature.

**[0025]** The present liposomal formulations provide a drug-delivery system which allows infusion of high concentrations of taxanes or other antineoplastic drugs in a stable form and which provides sustained therapeutic benefits at target sites, while maintaining low concentrations of insoluble free taxane and minimal adverse toxic effects than were previously known.

**[0026]** The present pharmaceutical composition can be administered in amounts of at least 50 to 400 mg of active compound/m$^2$ of mammalian host surface area, within a period of less than about three hours, preferably in less than about two hours, and most preferably 90 minutes without causing a substantial toxic reaction. For example, in a 70 kg human, about 0.5 to 7.0 mg active compound per kg of body weight can be safely administered in about 90 minutes. Preferably, about 1.0-5.0 mg of active compound per kg of body weight is administered. Alternatively, preferable amounts include 75, 135, 175, 250, 300,325, and 375 mg/m$^2$.

**[0027]** The present liposome compositions can be administered intravenously, intraperitoneally, to an isolated portion of a mammalian body, particularly a human body, such as an arm or leg, or in the case of a human, a hand, or can be injected directly into a tumor. Preferrably the formulations of the present invention can be in injectable form.

**[0028]** Liposomal encapsulated taxane has a substantial beneficial effect in overcoming multidrug resistance in cancer cells, which are subjected to chemotherapy. By using the liposomal composition of the present invention, it is possible to reduce the tendency of cancer cells subjected to chemotherapy to develop resistance to the chemotherapeutic agents used for chemotherapy such as anthracycline glycosides. This method includes administering to a host a pharmaceutical composition of a liposomal encapsulated taxane of the present invention in accordance with the administration protocol.

**[0029]** Taxanes and the anti-neoplastic derivatives thereof may be used to treat any form of mammalian cancer. Such compounds are thought to function by promoting the assembly of microtubules or prohibiting the tubulin disassembly process. Taxane and the anti-neoplastic derivatives thereof are of particular advantageous use in the treatment of mammalian lymphoma, ovarian, breast, lung and colon cancer, and particularly those conditions in humans.

**[0030]** The following examples further illustrate the present invention but, of course, should not be construed as in any way limiting its scope.

## EXAMPLE 1

**[0031]** This example demonstrates the construction of a liposomal encapsulated taxane formulation of the present invention. The following ingredients were employed in the amounts as indicated in Table 1:

**Table 1**

| Ingredients | Concentration | Concentration |
|---|---|---|
| Taxane | 2.0 mg (paclitaxel) | 4.0 mg (docetaxel) |
| 1,2-Dioleoyl-sn-Glycero-3-Phosphocholine (DOPC) | 54.2 mg | 57.2 |
| Tetramyristoyl Cardiolipin (CL) | 4.9 mg | 5.2 mg |
| Cholesterol (CH) | 1.48 mg | 1.6 mg |
| D-Alpha Tocopheryl acid Succinate | 0.31 mg | 0.31 mg |
| Sucrose | 100 mg | 200 mg |
| Sodium Chloride (NaCl) | 9.0 mg | 9.0 mg |
| Methylene Chloride, USP* | 1130 mg | -- |
| Dehydrate alcohol, USP* | -- | 158 mg |
| Water for Injection QS. AD. | 1.0 ml | 1.0 ml |

*To be removed during evaporation and lyophilization processes.

**[0032]** The lipids (DOPC, 1,2-dimyristoyl cardiolipin, cholesterol and D-Alpha Tocopheryl acid Succinate) and paclitaxel or docetaxel were dissolved in the methylene chloride or dehydrate alcohol. The lipid solution then was evaporated to dryness using a rotary evaporator under vacuum. After evaporation, the lipid residue was further dried overnight in a dessicator. The sucrose and NaCl were dissolved in de-ionized water to achieve the required batch concentrations. Then, the dried lipid residue was hydrated in the sucrose/NaCl solution to form multi-lamellar vesicles (MLV). The size of the MLV was further reduced in size by extrusion through 0.8, μm, 0.4, μm, 0.2 μm, and 0.1 μm sized polycarbonate filters. Five millimeters of the final formulation was filled into glass vials and freeze-dried using a benchtop VIRTIS Lyophilizer.

## EXAMPLE 2

**[0033]** This example demonstrates the construction of a liposomal encapsulated taxane formulation of the present invention. The following ingredients were employed in the amounts as indicated in Table 2:

**Table 2**

| Ingredients | Concentration | Concentration |
|---|---|---|
| Taxane | 2.0 mg (paclitaxel) | 6.0 mg (docetaxel) |
| 1,2-Dioleoyl-sn-Glycero-3-Phosphocholine (DOPC) | 53 mg | 78 mg |
| Tetramyristoyl Cardiolipin (CL) | 7.5 mg | 8.4 mg |
| Cholesterol (CH) | -- | 10.2 mg |
| D-Alpha Tocopheryl acid Succinate | 0.43 mg | 0.31 mg |
| Sucrose | 100 mg | 200 mg |
| NaCl | 9.0 mg | 2.5 mg |
| Methylene Chloride, USP* | 1068 mg | -- |
| Dehydrate alcohol, USP* | - | 158 |
| Water for Injection QS. AD. | 1.0 ml | 1.0 ml |

*To be removed during evaporation and lyophilization processes.

**[0034]** This formulation was manufactured as indicated in Example 1.

## EXAMPLE 3

**[0035]** This example demonstrates the construction of a liposomal encapsulated taxane formulation of the present invention. The following ingredients were employed in the amounts as indicated in Table 3:

**Table 3**

| Ingredients | Concentration | Concentration |
|---|---|---|
| Taxane | 2.0 mg (paclitaxel) | 4.0 mg (docetaxel) |
| 1,2-Dioleoyl-sn-Glycero-3-Phosphocholine (DOPC) | 54.2 mg | 57.2 mg |
| Tetramyristoyl Cardiolipin (CL) | 4.9 mg | 5.2 mg |
| Cholesterol (CH) | 1.5 mg | 1.6 mg |
| D-Alpha Tocopheryl acid Succinate | 0.31 mg | 0.31 mg |
| Sucrose | 200 mg | 200 mg |
| NaCl | 9.0 mg | 9.0 mg |
| Dehydrate alcohol, USP* | 200 mg | 158 mg |
| Water for Injection QS. AD. | 1 ml | 1.0 ml |

*To be removed during evaporation and lyophilization processes

**[0036]** This formulation was manufactured as indicated in Example 1.

## EXAMPLE 4

**[0037]** This example demonstrates the properties of the formulation manufactured in accordance with Example 1.

**[0038]** The lyophilized vials were reconstituted with 4.5 mL of de-ionized water. After complete reconstitution, the liposomes were further diluted 1:8 in normal saline or deionized water. The liposome vesicle size of the reconstituted and diluted formulations was measured by dynamic light scattering technique (Nicomp Submicron Particle Sizer) over a 17-37 days period, wherein the reconstituted formulations were maintained at room temperature. The reconstituted and diluted formulations also were examined for presence of paclitaxel and docetaxel crystals using an optical microscope (DMIL Microscope).

**[0039]** The results of these measurements made from the paclitaxel and docetaxel formulations prior to lyophilization are presented in Table 4 and 5:

**Table 4**. Physical Stability of Liposomal Paclitaxel Formulation

| Time (Days) | Condition | Particle Size (nm) | | Optical Microscopic Observations |
|---|---|---|---|---|
| | | Mean | 99 Percentile < | |
| Initial | | 113 | 189 | No crystals |
| 1 | 2-8°C | 115 | 185 | No crystals |
| 2 | 2-8°C | 114 | 188 | No crystals |
| 3 | 2-8°C | 101 | 167 | No crystals |
| 4 | 2-8°C | 112 | 179 | No crystals |
| 7 | 2-8°C | 108 | 182 | No crystals |
| 17 | 2-8°C | 105 | 181 | No crystals |

**Table 5.** Physical Stability of Liposomal Docetaxel Formulation

| Time (Days) | Condition | Particle Size (nm) | | Optical Microscopic Observations |
|---|---|---|---|---|
| | | Mean | 99 Percentile < | |
| Initial | | 106 | 179 | No crystals |
| 2 | 2-8°C | 108 | 188 | No crystals |
| 5 | 2-8°C | 106 | 173 | No crystals |
| 9 | 2-8°C | 106 | 177 | No crystals |
| 12 | 2-8°C | 107 | 180 | No crystals |
| 16 | 2-8°C | 106 | 156 | No crystals |
| 37 | 2-8°C | 106 | 175 | No crystals |

**[0040]** The results of these measurements made from the paclitaxel and docetaxel formulations of Example 1 after lyophilization and reconstitution are presented in Table 6 and 7:

**Table 6**

Physical Stability of Liposomal Paclitaxel Formulation after lyophilization and reconstitution

| Time (Days) | Condition | Particle Size (nm) | | Optical Microscopic Observations |
|---|---|---|---|---|
| | | Mean | 99 Percentile < | |
| Initial | Room temp. | 134 | 283 | No crystals |
| 1 | Room temp. | 133 | 253 | No crystals |
| 3 | Room temp. | 131 | 260 | No crystals |
| 4 | Room temp. | 132 | 272 | No crystals |

**[0041]** The measurements made from the paclitaxel formulation of Example 1 after dilution (1:8 in normal saline) are set forth in Table 7:

**Table 7**

| Time (Days) | Condition | Particle Size (nm) | | Optical Microscopic Observations |
|---|---|---|---|---|
| | | Mean | 99 Percentile < | |
| Initial | Room temp. | 134 | 268 | No crystals |
| 1 | Room temp. | 135 | 259 | No crystals |
| 3 | Room temp. | 133 | 261 | No crystals |
| 4 | Room temp. | 131 | 273 | No crystals |

**[0042]** The measurements made from the docetaxel formulation of Example 1 after dilution (1:8 in normal saline) are set forth in Table 8:

**Table 8**

| Time after dilution (Hours) | Condition | Particle Size(nm) | | Optical Microscopic Observations |
|---|---|---|---|---|
| | | Mean | 99 Percentile < | |
| Before dilution | | 112 | 196 | No crystals |
| 0 | | 115 | 206 | No crystals |
| 6 | RT | 116 | 208 | No crystals |
| 24 | RT | 115 | 196 | No crystals |

## EXAMPLE 4

**[0043]** This example demonstrates the properties of the formulation manufactured in accordance with Example 2. The methodology is the same as that described in Example 3, and the measurements made from the paclitaxel and docetaxel formulation prior to lyophilization are set forth in Table 9 and 10:

**Table 9**

| Time (Days) | Condition | Particle Size (nm) | |
|---|---|---|---|
| | | Mean | 99 Percentile < |
| Initial | Room temp. | - | - |
| 1 | Room temp. | 109 | 175 |
| 2 | Room temp. | 109 | 185 |
| 3 | Room temp. | 112 | 172 |
| 4 | Room temp. | 111 | 165 |

**Table 10**

Physical stability of liposomal docetaxel formulation prior to lyophilization

| Time (Days) | Condition | Particle Size (nm) | | Optical Microscopic Observations |
|---|---|---|---|---|
| | | Mean | 99 Percentile < | |
| Initial | | 112 | 183 | No crystals |
| 2 | 2-8°C | 113 | 194 | No crystals |
| 5 | 2-8°C | 113 | 200 | No crystals |

**[0044]** The results of these measurements made from the formulations of Example 2 after lyophilization and reconstitution are presented in Table 11 and 12:

**Table 11**

| Time (Days) | Condition | Particle Size (nm) | | Optical Microscopic Observations |
|---|---|---|---|---|
| | | Mean | 99 Percentile < | |
| Initial | Room temp. | 148 | 317 | No crystals |
| 1 | Room temp. | 142 | 308 | No crystals |
| 2 | Room temp. | 146 | 310 | No crystals |
| 3 | Room temp. | 142 | 293 | No crystals |
| 4 | Room temp. | 144 | 314 | NA |

**Table 12**

Physical stability of liposomal docetaxel formulation after lyophilization and reconstitution

| Time after reconstitution (Hours) | Condition | Particle Size(nm) | | Optical Microscopic Observations |
|---|---|---|---|---|
| | | Mean | 99 Percentile < | |
| Before lyo | | 124 | 208 | No crystals |
| 0 | | 129 | 275 | No crystals |
| 4 | RT | 136 | 280 | No crystals |
| 7 | RT | 135 | 283 | No crystals |
| 24 | 2-8°C | 137 | 280 | No crystals |
| 72 | 2-8°C | 138 | 293 | No crystals |

**[0045]** The measurements made from the formulation of Example 2 after dilution (1:8 in normal saline) are set forth in Table 13:

**Table 13**

| Time (Days) | Condition | Particle Size (nm) | | Optical Microscopic Observations |
|---|---|---|---|---|
| | | Mean | 99 Percentile < | |
| Initial | Room temp. | 147 | 305 | No crystals |
| 3 | Room temp. | 142 | 302 | No crystals |
| 4 | Room temp. | 145 | 311 | No crystals |

## EXAMPLE 5

**[0046]** This example demonstrates the entrapment efficiency of the formulation manufactured in accordance with Example 3. The drug entrapment efficiency was determined by size exclusion column chromatography using SEPHADAX G-50 column. The drug and lipid contents were determined by HPLC methods.

**Table 14**

| Time (Days) | Condition | Entrapment Efficiency (%) |
|---|---|---|
| Initial | Room temp. | >90 |
| 1 | Room temp. | >90 |
| 2 | Room temp. | >90 |
| 3 | Room temp. | >90 |

**Table 15**

| Time (Days) | Condition | Entrapment Efficiency (%) |
|---|---|---|
| Initial | Refrigerator temp. | >90 |
| 1 | Refrigerator temp. | >90 |
| 2 | Refrigerator temp. | >90 |
| 3 | Refrigerator temp. | >90 |

## EXAMPLE 6

**[0047]** This example presents a comparative multiple IV dose toxicity study of Taxol and Liposome Encapsulated Paclitaxel (LEP).

## MATERIALS AND METHODS

### Test System

**[0048]** CD2F1 mice (4-6 weeks old, Male and Female) used for the study were obtained through Harlan Sprague Dawley Laboratories. The individual animal was identified by ear tag. Upon arrival, the animals were placed in quarantine for 7 days. The animals were kept in environmentally monitored, well-ventilated room maintained at a temperature of 64-84 °F and a relative humidity of 30%-70%. Fluorescent lighting provided illumination approximately 12 hours per day. Mice were offered ad libitum 8656 HT Rodent Diet (Harlan Teklad, Madison, WI) during the quarantine and study periods. The average weight of mice on day 1 of study was 16-22 g (female) and 20-27 (male). The age of mice on day 1 of study was 6-7 weeks.

**Test and Control Articles**

**Test Articles:**

1. Lyophilized Liposomal Paclitaxel (LEP-ETU) vials prepared in accordance with the present invention were stored at 2-8 °C.

2. Taxol vials (30 mg/vial; concentration 6 mg/mL) were received from Mead Johnson, Inc.

**Control Article:**

1. Placebo liposome was stored at 2-8 °C.

**Dose Formulations Preparation:**

**[0049]** Vehicle formulations (LEP and Placebo liposome) were prepared fresh on each day of dosing. The stability of reconstituted and diluted formulations (up to 8 fold with 0.9% saline) is 12 hours at 20-25 °C. All the dosing solutions were used within 12 hours after reconstitution and dilutions.

**EXPERIMENTAL DESIGN**

**Randomization and Group Assignment**

**[0050]** Randomization was done during week -1. Animals were weighed prior to randomization and only the animals whose weight ranged between 16-23 g (Female) and 17-26 g (Male) were used for randomization and were assigned to the following groups (7 animals/sex/group). The randomization is presented in Table 16:

**Table 16**

| Group Number | Treatment | Dose Level (mg/kg/day) | Dose Concentration (mg/mL) | Dose volume (mL/Kg/dose) |
|---|---|---|---|---|
| 1 | Placebo Liposome | 0.0 | 0.0 | 25 |
| 2 | LEP | 25.0 | 1.0 | 25 |
| 3 | LEP | 50.0 | 2.0 | 25 |
| 4. | Cremophor EL/Ethanol | 0.0 | 0.0 | 25 |
| 5. | Cremophor EL/Ethanol | 0.0 | 0.0 | 25 |
| 6 | Taxol | 12.5 | 0.5 | 25 |
| 7 | Taxol | 25.0 | 1.0 | 25 |

**Article (Control and Test) Administration:**

**[0051]** Each animal was weighed prior to dosing. Mice received control or test article intravenously via tail vein once a day for 5 consecutive days. The injection volume was based on individual mouse body weight. The control animals in Group 1 received approximately the same amount of lipids as in 50 mg/kg dose of LEP. The

control animals in Groups 4 and 5 received the equivalent amount of Cremophor EL/Ethanol as in Group 6 and 7, respectively but without Paclitaxel.

**Observations**

**[0052]** Animals were weighed daily during the dosing period (Day 1-5) and thereafter three times a week for up to 22 days. Animals were observed once daily during the study period for morbidity / mortality. Animals were observed approximately 1 to 2 hours post dosing for clinical signs and daily thereafter. Detailed physical examination for toxicity was done on Day 1 and thereafter once a week.

**RESULTS**

**Body Weights**

**[0053]** There was no body weight loss in animals that received placebo liposome. Female mice given IV doses of LEP (25 mg/kg/dose) lost weight by 5.2% on Day 8 and recovered completely by Day 10. There was a 12.5% body weight loss for male mice that received LEP (25 mg/kg/dose) by Day 8 and the complete recovery was achieved by Day 15. The weight loss for male and female mice in Group 3 (50 mg/kg/dose, LEP) were 20.6 and 28.7% by Day 8. The control animals that were injected with Cremophor EL/Ethanol had no body weight loss. The weight loss for females in Group 5 (12.5 mg/kg/dose, Taxol) was negligible. The male animals in Group 5 had a body weight loss of 4.7% by Day 5 and the recovery was complete by Day 12. Female animals in Group 7 (25 mg/kg/dose, Taxol) had a body weight loss of 6.3% by Day 8 and they recovered completely from body weight loss by Day 12. The weight loss for male animals in Group 7 was 10.4% on Day 8 and the recovery was complete by Day 15. The percentage of survival (Total/Number surviving) is presented in Tables 17 and 18. On Day 4, one male animal in Group 7 (25 mg/kg/dose, Taxol) died 1h after administration of article. One animal in Group 4 was sacrificed on Day 17 because of wounded tail.

**Table 17.**

A Comparative Multiple IV Dose Toxicity Study of Taxol and LEP: Percentage of Survival of Female animals (Number surviving/Total)

| Day | Group 1 Female | Group 2 Female | Group 3 Female | Group 4 Female | Group 5 Female | Group 6 Female | Group 7 Female |
|---|---|---|---|---|---|---|---|
| 1 | 100(7/7) | 100(7/7) | 100(7/7) | 100(7/7) | 100(7/7) | 100(7/7) | 100(7/7) |
| 2 | 100(7/7) | 100(7/7) | 100(7/7) | 100(7/7) | 100(7/7) | 100(7/7) | 100(7/7) |
| 3 | 100(7/7) | 100(7/7) | 100(7/7) | 100(7/7) | 100(7/7) | 100(7/7) | 100(7/7) |
| 4 | 100(7/7) | 100(7/7) | 100(7/7) | 100(7/7) | 100(7/7) | 100(7/7) | 100(7/7) |
| 5 | 100(7/7) | 100(7/7) | 100(7/7) | 100(7/7) | 100(7/7) | 100(7/7) | 100(7/7) |
| 6 | 100(7/7) | 100(7/7) | 100(7/7) | 100(7/7) | 100(7/7) | 100(7/7) | 100(7/7) |
| 7 | 100(7/7) | 100(7/7) | 100(7/7) | 100(7/7) | 100(7/7) | 100(7/7) | 100(7/7) |
| 8 | 100(7/7) | 100(7/7) | 100(7/7) | 100(7/7) | 100(7/7) | 100(7/7) | 100(7/7) |
| 9 | 100(7/7) | 100(7/7) | 0(7/7) | 100(7/7) | 100(7/7) | 100(7/7) | 100(7/7) |
| 10 | 100(7/7) | 100(7/7) | 0(7/7) | 100(7/7) | 100(7/7) | 100(7/7) | 100(7/7) |
| 11 | 100(7/7) | 100(7/7) | 0(7/7) | 100(7/7) | 100(7/7) | 100(7/7) | 100(7/7) |
| 12 | 100(7/7) | 100(7/7) | 0(7/7) | 100(7/7) | 100(7/7) | 100(7/7) | 100(7/7) |
| 13 | 100(7/7) | 100(7/7) | 0(7/7) | 100(7/7) | 100(7/7) | 100(7/7) | 100(7/7) |
| 14 | 100(7/7) | 100(7/7) | 0(7/7) | 100(7/7) | 100(7/7) | 100(7/7) | 100(7/7) |
| 15 | 100(7/7) | 100(7/7) | 0(7/7) | 100(7/7) | 100(7/7) | 100(7/7) | 100(7/7) |
| 16 | 100(7/7) | 100(7/7) | 0(7/7) | 100(7/7) | 100(7/7) | 100(7/7) | 100(7/7) |
| 17 | 100(7/7) | 100(7/7) | 0(7/7) | 100(7/7) | 100(7/7) | 100(7/7) | 100(7/7) |
| 18 | 100(7/7) | 100(7/7) | 0(7/7) | 100(7/7) | 100(7/7) | 100(7/7) | 100(7/7) |
| 19 | 100(7/7) | 100(7/7) | 0(7/7) | 100(7/7) | 100(7/7) | 100(7/7) | 100(7/7) |
| 20 | 100(7/7) | 100(7/7) | 0(7/7) | 100(7/7) | 100(7/7) | 100(7/7) | 100(7/7) |
| 21 | 100(7/7) | 100(7/7) | 0(7/7) | 100(7/7) | 100(7/7) | 100(7/7) | 100(7/7) |
| 22 | 100(7/7) | 100(7/7) | 0(7/7) | 100(7/7) | 100(7/7) | 100(7/7) | 100(7/7) |

Group 1: Placebo liposome equivalent to lipid amount present in the LEP dose of 50 mg/kg

Group 2: LEP 25 mg/kg;

Group 3: LEP 50 mg/kg

Group 4: Cremophor EL/Ethanol equivalent to cremophor EL/Ethanol present in Taxol dose of 12.5 mg/kg

Group 5: Cremophor EL/Ethanol equivalent to cremophor EL/Ethanol present in Taxol dose of 25 mg/kg

Group 6: Taxol 12.5 mg/kg

Group 7: Taxol 25 mg/kg

**Table 18.**

A Comparative Multiple IV Dose Toxicity Study of Taxol and LEP: Percentage of Survival of Male animals (Number surviving/Total)

| Day | Group1 Male | Group 2 Male | Group 3 Male | Group 4 Male | Group 5 Male | Group 6 Male | Group 7 Male |
|---|---|---|---|---|---|---|---|
| 1 | 100(7/7) | 100(7/7) | 100(7/7) | 100(7/7) | 100(7/7) | 100(7/7) | 100(7/7) |
| 2 | 100(7/7) | 100(7/7) | 100(7/7) | 100(7/7) | 100(7/7) | 100(7/7) | 100(7/7) |
| 3 | 100(7/7) | 100(7/7) | 100(7/7) | 100(7/7) | 100(7/7) | 100(7/7) | 100(7/7) |
| 4 | 100(7/7) | 100(7/7) | 100(7/7) | 100(7/7) | 100(7/7) | 100(7/7) | 86(6/7) |
| 5 | 100(7/7) | 100(7/7) | 86(6/7) | 100(7/7) | 100(7/7) | 100(7/7) | 86(6/7) |
| 6 | 100(7/7) | 100(7/7) | 71.4(5/7) | 100(7/7) | 100(7/7) | 100(7/7) | 86(6/7) |
| 7 | 100(7/7) | 100(7/7) | 57.1(4/7) | 100(7/7) | 100(7/7) | 100(7/7) | 71(5/7) |
| 8 | 100(7/7) | 100(7/7) | 0(7/7) | 100(7/7) | 100(7/7) | 100(7/7) | 71(5/7) |
| 9 | 100(7/7) | 100(7/7) | 0(7/7) | 100(7/7) | 100(7/7) | 100(7/7) | 57(4/7) |
| 10 | 100(7/7) | 100(7/7) | 0(7/7) | 100(7/7) | 100(7/7) | 100(7/7) | 57(4/7) |
| 11 | 100(7/7) | 100(7/7) | 0(7/7) | 100(7/7) | 100(7/7) | 100(7/7) | 57(4/7) |
| 12 | 100(7/7) | 100(7/7) | 0(7/7) | 100(7/7) | 100(7/7) | 100(7/7) | 57(4/7) |
| 13 | 100(7/7) | 100(7/7) | 0(7/7) | 100(7/7) | 100(7/7) | 100(7/7) | 57(4/7) |
| 14 | 100(7/7) | 100(7/7) | 0(7/7) | 100(7/7) | 100(7/7) | 100(7/7) | 57(4/7) |
| 15 | 100(7/7) | 100(7/7) | 0(7/7) | 100(7/7) | 100(7/7) | 100(7/7) | 57(4/7) |
| 16 | 100(7/7) | 100(7/7) | 0(7/7) | 100(7/7) | 100(7/7) | 100(7/7) | 57(4/7) |
| 17 | 100(7/7) | 100(7/7) | 0(7/7) | 86(6/7) | 100(7/7) | 100(7/7) | 57(4/7) |
| 18 | 100(7/7) | 100(7/7) | 0(7/7) | 86(6/7) | 100(7/7) | 100(7/7) | 57(4/7) |
| 19 | 100(7/7) | 100(7/7) | 0(7/7) | 86(6/7) | 100(7/7) | 100(7/7) | 57(4/7) |
| 20 | 100(7/7) | 100(7/7) | 0(7/7) | 86(6/7) | 100(7/7) | 100(7/7) | 57(4/7) |
| 21 | 100(7/7) | 100(7/7) | 0(7/7) | 86(6/7) | 100(7/7) | 100(7/7) | 57(4/7) |
| 22 | 100(7/7) | 100(7/7) | 0(7/7) | 86(6/7) | 100(7/7) | 100(7/7) | 57(4/7) |

Group 1: Placebo liposome equivalent to lipid amount present in the LEP dose of 50 mg/kg;

Group 2: LEP 25 mg/kg;

Group 3: LEP 50 mg/kg;

Group 4: Cremophor EL/Ethanol equivalent to cremophor EL/Ethanol present in Taxol dose of 12.5 mg/kg;

Group 5: Cremophor EL/Ethanol equivalent to cremophor EL/Ethanol present in Taxol dose of 25 mg/kg;

Group 6: Taxol 12.5 mg/kg;

Group 7: Taxol 25 mg/kg.

**Clinical Signs of Toxicity**

**[0054]** Clinical signs of toxicity of animals in each group on various days is presented in Table 19. Clinical signs of toxicity as manifested by hunched posture and rough coats were observed in animals in Group 7 (25 mg/kg/dose, Taxol) starting from Day 4. Starting from Day 9 till Day 15, the animals in Group 7 showed neurological signs of toxicity manifested by problems in walking and dragging the back legs towards abdomen. On Day 6, four male animals in Group 6 (12.5 mg/kg/dose, Taxol) had rough coats and three were dehydrated but by Day 7, they appeared to be normal. Animals in Group 3 (50 mg/kg/dose LEP), had severe signs of toxicity as manifested by hunched posture, dehydration and rough coats between Day 6-9 and the animals in this group were either dead or moribund sacrificed by Day 9.

**Clinical Observations**

**[0055]** The detailed physical for toxicity was done on Days 1, 10, 17, and 22 and is presented in Table 20.

**Unscheduled Observations**

**[0056]** All the animals in Group 5 receiving a dose of Cremophor EL/Ethanol equivalent to that present in 25 mg/kg/dose of Taxol and Group 7 (25 mg/kg/dose, Taxol) had decreased activity after dosing. All the unscheduled observations after dosing are presented in a tabular form (Table 21).

**CONCLUSIONS**

**[0057]** The tolerated doses for LEP was 25 mg/kg/day (once daily x 5 days, whereas for Taxol, a dose of 12.5 mg/kg/day (once daily x 5 days) could be administered safely to CD2F1 mice.

**Table 19**

A Comparative Multiple IV dose Toxicity Study of Taxol (Paclitaxel) and LEP in CD2F1 mice: Animal Clinical Signs for Toxicity on Different Days

| Day (s) | Clinical Signs |
|---|---|
| 1-2 | Animals in all groups were normal. |
| 3 | Animals in Groups 1-6 were normal. All animals in Group 7 had rough coats and hunched posture. |
| 4 | Animals in Group 1-6 were normal. Animals in Group 7 had rough coats and hunched posture. |
| 5 | Animals in Groups 1-6 were normal. All animals in Group 7 had rough coats. |
| 6 | All animals in Group 1 were normal. Four female animals in Group 2 (ID# 16, 17, 37, 45) were dehydrated and had rough coats. One female animal in Group 2 (ID# 38) had a rough coat. Six male animals in Group 2 (ID# 76, 77, 133, 134, 90, 125) were dehydrated and had rough coats. One male animal in Group 2 (ID# 128) was dehydrated. Three females in Group 3 (ID# 23, 24, and 31) had rough coats, piloerection, hunched posture, and were dehydrated. Four females in Group 3 (ID# 33, 3, 4, and 10) had hunched posture, Piloerection and were dehydrated. Two males in Group 3 (ID# 110 and 119) had rough coats, hunched posture and were dehydrated. Two males in Group 3 (ID# 85 and 127) had rough coats, piloerection, hunched posture and were dehydrated. One male in Group 3 (ID# 108) had a rough coat, hunched posture, piloerection, swollen tongue, and was dehydrated. One male in Group 3 (ID# 118) had a rough coat, hunched posture, swollen tongue, swollen eye, and was dehydrated. All females in Group 4 were normal. All males in Group 4 were normal, except (ID# 100) which had a scab on its tail. All animals in Group 5 were normal except one male animal (ID# 99) which had a rough coat. All females in Group 6 and three males in Group 6 (ID# 86, 98, and 124) were normal. Three males in Group 6 (ID# 79, 93, and 137) were dehydrated and had rough coats. One male animal in Group 6 (ID#96) was dehydrated. Except one animal (ID#11 who was normal), all females in Group 7 were dehydrated. All male animals in Group 7 were dehydrated and had rough coats. Five male animals in Group 7 (ID# 81, 88, 116, 131, and 142) had hunched posture. Four male animals in Group 7 (ID# 81, 88, 131, 142) appeared to have lump in the upper, left abdominal area. |
| 7 | All animals in Groups 1, 2, 4, 5 and 6 were normal. All females in Group 7 were normal. All female animals in Group 3 had decreased activity, and rough coats. All male animals in Group 3 had decreased activity. All female animals in Group 7 were normal. All male animals in Group 7 had rough coats. |

**Table 19 (continued)**

| Day (s) | Clinical Signs |
|---|---|
| 8 | All animals in Groups 1, 2, 5 and 6 were normal. All females in Groups 4 and 7 were normal. All female animals in Group 3 had rough coats and hunched posture. All males in Group 3 were dehydrated, had rough coats, hunched posture and decreased activity. All male animals in Group 4 were normal except one male (ID#100) had an open wound on its tail. All male animals in Group 7 had rough coats. |
| 9 | All animals in Groups 1, 2, 5 and 6 were normal. All females in Groups 4 and 7 were normal. All female animals in Group 3 had rough coats and hunched posture. Three female animals in Group 3 (ID# 23, 24, 31) were also dehydrated. Two female animals in Group 3 (ID# 3, 4) had lameness in the back and front legs. One animal in Group 3 (ID# 10) had lameness in the back legs. All male animals in Group 4 were normal except one male (ID# 100) had a damaged tail. All male animals in Group 7 had rough coats. One male animal in Group 7 (ID# 142) was also dehydrated, had hunched posture and lameness in the back legs. |
| 10 | All animals in Groups 1, 2, 5 and 6 were normal. All female animals in Group 4 were normal. One male animal in Group 4 (ID# 100) had a damaged tail, all other males in this group were normal. All animals in Group 7 were ataxic. |
| 11 | All animals in Groups 1, 2, 5 and 6 were normal. All female animals in Group 4 were normal. All male animals in Group 4 were normal except one male animal (ID# 100) had damaged tail. All female animals in Group 7 had piloerection. All male animals in Group 7 had Piloerection, except one male animal (ID# 113) had a rough coat. |
| 12 | All animals in Group 1 were normal. All female animals in Groups 2, 4 and 6 were normal. All male animals in Group 2 were dehydrated. Six male animals in Group 2 (ID# 76, 77, 133, 134, 90, and 125) were ataxic. Six male animals in Group 4 (ID# 122, 135, 114, 100, 112, and 129) were dehydrated. Three male animals in Group 4 (ID# 100, 112, 129) had rough coats. One male animal in Group 4 (ID# 100) had a damaged tail. All females in Group 5 were normal, except one animal (ID# 13) was dehydrated. One male animal in Group 5 (ID# 120) was normal. Six male animals in Group 5 (ID# 87, 89, 123, 80, 99, and 136) were dehydrated. One male in Group 5 (ID# 80) had a rough coat. All male animals in Group 6 were normal, except two male animals (ID# 86, 79) were dehydrated. All animals in Group 7 were ataxic. Two female animals in Group 7 (ID# 49 and 47), and all males in Group 7 were also dehydrated. |
| 13 | All animals in Groups 1, 2, 5 and 6 were normal. All female animals in Group 4 were normal. All males in Group 4 were normal, except one male animal (ID# 100) had a damaged tail. The animal # 100 had damaged tail. All animals in Group 7 were ataxic. |
| 14 | All animals in Groups 1, 2, 5 and 6 were normal. All female animals in Group 4 and 7 were normal. Two male animals in Group 4 (ID# 100 and 129) had rough coats. All male animals in Group 7 were ataxic. Three male animals in Group 7 (ID# 81, 116, and 113) were dehydrated. One male animal in Group 7 (ID# 113) had a rough coat. |

**Table 19 (continued)**

| Day (s) | Clinical Signs |
|---|---|
| 15 | All animals in Groups 1, 2, 5 and 6 were normal. All female animals in Group 4 and 7 were normal. One male animal in Group 4 (ID# 100) had a damaged tail. All male animals in Group 7 were ataxic. |
| 16 | All animals in Groups 1, 2, 5, and 6 were normal. All female animals in Groups 4 and 7 were normal. One male animal in Group 4 (ID# 100) had a damaged tail. All male animals in Group 7 were ataxic. |
| 17 | All animals in Groups 1, 2, 5, 6, and 7 were normal. All female and male animals in Group 4 were normal, except one male animal (ID# 100) had a severely damaged tail. |
| 18-21 | Animals in all Groups were normal. |
| 22 | Animals in Groups 1, 2 and 7 were normal. All female animals in Groups 4, 5, and 6 were normal. One male animal in Group 4 (ID# 129), two male animals in Group 5 (ID# 123, 136) and one male in Group 6 (ID# 79) were dehydrated. |

Group 1: Placebo liposome equivalent to lipid amount present in the LEP dose of 50 mg/kg

Group 2: LEP 25 mg/kg;

Group 3: LEP 50 mg/kg

Group 4: Cremophor EL/Ethanol equivalent to cremophor EL/Ethanol present in Taxol dose of 12.5 mg/kg

Group 5: Cremophor EL/Ethanol equivalent to cremophor EL/Ethanol present in Taxol dose of 25 mg/kg

Group 6: Taxol 12.5 mg/kg;

Group 7: Taxol 25 mg/kg

**Table 20**

A Comparative Multiple IV dose Toxicity Study of Taxol (Paclitaxel) and LEP in CD2F1 mice: Clinical observations

| Day (s) | Clinical Observations |
|---|---|
| 1 | Animals in all groups were normal. |
| 10 | All animals in Groups 1 and 5 were normal. Three female animals in Group 2 (ID# 14, 15 and 16) were normal. Three female animals in Group 2 (ID# 17, 37 and 45) had lumps in the upper left abdominal area. One female animal in Group 2 (ID# 38) was ataxic. All male animals in Group 2 were normal, except one male animal (ID# 125) had lump in the upper left abdominal area. Six female animals in Group 4 (ID# 2, 9, 44, 22, 25 and 46) were normal. One female animal in Group 4 (ID# 32) had lump in the upper left abdominal area. Six male animals in Group 4 (ID# 122, 126, 135, 114, 112 and 129) were normal. One male animal in Group 4 (ID# 100) had severely damaged tail. Five female animals in Group 6 (ID# 27, 42, 20, 21 and 41) were normal. One female animal in Group 6 (ID# 12) had lump in the upper left abdominal area. One female animal in Group 6 (ID#40) was dehydrated. All male animals in Group 6 were normal, except two male animals (ID#124 and 79) were dehydrated. All animals in Group 7 were ataxic and dehydrated. Two female animals (ID# 28, 30) and one male animal (ID# 113) in Group 7, had lump in the upper left abdominal area. Three male animals in Group 7 (ID# 88,116 and 113) had rough coats. One male animal in Group 7 (ID#88) had hunched posture. |
| 17 | All animals in Groups 1 and 2 were normal. All female and male animals in Group 4 were normal, except one male animal (ID# 100) had a necrotic tail. All female animals in Group 5 were normal. Four male animals in Group 5 (ID# 87, 120, 123 and 136) were dehydrated. Three male animals in Group 5 (ID# 89, 80 and 99) were normal. All animals in Group 6 were normal, except one male animal (ID#79) was dehydrated. All female animals in Group 7 were normal. Three male animals in Group 7 (ID# 81, 88 and 113) had uncoordinated movements in left hind leg. Three male animals in Group 7 (ID# 88, 116 and 113) were dehydrated. |
| 22 | Animals in Groups 1, 2 and 7 were normal. All animals in Group 4 were normal, except one male animal (ID#129) was dehydrated. All animals in Group 5 were normal except two male animals (ID#123 and 136) were dehydrated. All animals in Group 6 were normal, except one male animal (ID# 79) was dehydrated. |

Group 1: Placebo liposome equivalent to lipid amount present in the LEP dose of 50 mg/kg;

Group 2: LEP 25 mg/kg; Group 3: LEP 50 mg/kg;

Group 4: Cremophor EL/Ethanol equivalent to cremophor EL/Ethanol present in Taxol dose of 12.5 mg/kg;

Group 5: Cremophor EL/Ethanol equivalent to cremophor EL/Ethanol present in Taxol dose of 25 mg/kg;

Group 6: Taxol 12.5 mg/kg; Group 7: Taxol 25 mg/kg

**Table 21**

A comparative multiple IV dose toxicity study of LEP and Taxol: Unscheduled observations: Unscheduled Observations

| Day | Unscheduled Observations |
|---|---|
| 1 | All animals in Groups 5 and 7 were in comatose after dosing. One female animal in Group 6 (ID#20) had decreased activity after dosing. |
| 2 | All animals in Groups 5 and 7 and one animal in Group 7 (ID# 137) were in comatose after dosing. |
| 3 | All animals in Groups 6 and 7 appeared to have decreased activity after dosing. One male animal (#83) and one female animal (#1) in Group 1 had scab on tail. |
| 4 | All animals in Groups 6 and 7 appeared to have decreased activity after dosing. One male animal in Group 3 (ID#85) had a wound on its tail. One male in Group 4 (ID#100) had multiple wounds on its tail. Two male animals (ID# 83 and 117) appeared to have a scab on their tails. One male animal in Group 1 (ID#132) had tail sheet coming off the base of its tail. |
| 5 | One male animal in Group 1(ID#130) died after dosing. One male animal in Group 2 (ID#125) had multiple wounds on its tail. One male animal in Group 1 (ID#132) had scab on its tail. |

Group 1: Placebo liposome equivalent to lipid amount present in the LEP dose of 50 mg/kg

Group 2: LEP 25 mg/kg;

Group 3: LEP 50 mg/kg

Group 4: Cremophor EL/Ethanol equivalent to cremophor EL/Ethanol present in Taxol dose of 12.5 mg/kg

Group 5: Cremophor EL/Ethanol equivalent to cremophor EL/Ethanol present in Taxol dose of 25 mg/kg

Group 6: Taxol 12.5 mg/kg;

Group 7: Taxol 25 mg/kg

## EXAMPLE 7

**[0058]** This example presents the results of a Therapeutic efficacy evaluation of liposome based formulation taxol (LEP) in SCID mice bearing human lung tumor (A549).

## MATERIALS AND METHODS

### Cell line and Culture condition:

**[0059]** The lung adenocarcinoma cell line A-549 was obtained from the American Type Culture Collection (Rockville, MD) and maintained in RPMI-1640 medium (Life Technologies Inc., Grand island, NY) supplemented with 10% heat inactivated fetal bovine serum (Life Technologies Inc., Grand island, NY). The cell line was grown at 37 °C in a humidified 5% $CO_2$ incubator.

### Mice:

**[0060]** C.B.-17 SCID female mice (3-4 weeks old) were received from Harlan Sprague Dawley (Indianapolis, IN). Mice were handled aseptically and housed in micro isolator in accordance with standard operating procedures (SOPs) of NeoPharm Research and Development, and received sterile food and water ad libitum. Mice were acclimated for at least five days before initiating the study.

### Drug and Formulation:

**[0061]** Taxol was obtained from MeadJohonson (Lot #IL5302). Liposome entrapped taxol and blank liposome were obtained as described above.

### Tumor Transplantation:

**[0062]** Logarithmically grown A549 cells suspension 50 x106 /mL was prepared and mice were transplanted with 5x106 cells (0.1mL) subcutaneously at left flank region (6). Tumor growth was measured with digital caliper (Mitutoyo Corporation, Japan) and tumor volume was determined by using formula: [length x (width/2) 2x p].

### Experimental Design:

**[0063]** After appropriate growth of tumors (23 days) animals were randomly divided into different treatment groups (5-7 animals/group) and treated with LEP (12.5 or 25 50 mg/kg x 3) or taxol (12.5 or 25.0 mg/kg x 3) or blank liposome or cremophor -EL on day 1, 4, and 8. Tumor growth inhibition was monitored till day 28 of post treatment.

**RESULTS AND DISCUSSION:**

**[0064]** Antitumor efficacy was evaluated in established human lung tumor xenograft model implanted s.c. in SCID mice. The efficacy of LEP and taxol was determined in multiple dose intravenous injection on day 1,4 and 8.. Animal group treated with taxol at 12.5 and 25.0 mg/kg resulted in 37 % and 57 % respectively compared to blank liposome treated groups.(Figure 1). However, inhibition of tumor growth was much more pronounced in animal groups treated with LEP. LEP treatment at 12.5 , 25.0 and 50 mg/kg resulted in 44%, 85% and 95% compared to the control group.

**[0065]** Both LEP and taxol were well tolerated at all the doses tested as judged by body weight loss (Figure 2). Only less than 5% body weight loss was noticed in case of LEP 50 mg/kg treated animal group.

**[0066]** Higher tumor growth inhibition of LEP treated animals could be due to the optimal availability of taxol at target site for longer period of time. Liposome based formulation of taxol has two advantages; 1) it helps to deliver the drug without using any surfactants and 2) provide sustained level of drug for longer period of time to exert its antitumor effects.

**[0067]** All of the references cited herein, including patents, patent applications, and publications, are hereby incorporated in their entireties (SP) by reference.

**[0068]** While this invention has been described with an emphasis upon preferred embodiments, it will be obvious to those of ordinary skill in the art that variations of the preferred embodiments may be used and that it is intended that the invention may be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications encompassed within the spirit and scope of the invention as defined by the following claims.

WE CLAIM:

1. A formulation of one or more antineoplastic drugs encapsulated in liposomes including at least a lipid fraction in addition to the antineoplastic drug, wherein the composition is stable in an aqueous solution at room temperature for at least 3 days.

2. The formulation of claim 1, which is substantially free of antineoplastic drug crystal and precipitate.

3. The formulation of claim 1, which is free of antineoplastic drug crystal and precipitate.

4. The formulation of any of claims 1-3, wherein an antineoplastic drug is a taxane or related compound.

5. The formulation of claim 4, wherein an antineoplastic drug is an epothilone A, an epothilone B, or derivative thereof.

6. The formulation of claim 4, wherein an antineoplastic drug is paclitaxel.

7. The formulation of any of claims 1-6, wherein at least about 85% of the antineoplastic drug is entrapped in the liposomes.

8. The formulation of any of claims 1-7, wherein the ratio of lipid to the antineoplastic drug is between about 10:1 and about 70:1 by molar ratio of the composition.

9. The formulation of any of claims 1-8, wherein the lipid fraction includes one or more lipids selected from the group consisting of 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), tetramyristoyl cardiolipin (CL), and cholesterol (CH).

10. The formulation of claim 9, wherein the lipid fraction includes two or more lipids selected from the group consisting of DOPC, CL, and CH.

11. The formulation of claim 9 or 10, wherein the mole percent ratio of DOPC:CH:CL is between about 92:0:8 and about 90:5:5.

12. The formulation of any of claims 9-11, wherein the lipid fraction includes DOPC, CL, and CH.

13. The formulation of any of claims 1-12, further comprising one or more antioxidants.

14. The formulation of claim 13, wherein an antioxidant is D-alpha tocopheryl acid succinate.

15. The formulation of any of claims 1-14, further comprising a lyoprotectant.

16. The formulation of claim 15 wherein a lyoprotectant includes one or more protective sugars selected from the group consisting of trehalose, maltose, sucrose, glucose, lactose, and dextran.

17. The formulation of claim 15 or 16 wherein a lyoprotectant is sucrose.

18. The formulation of any claims 1-17, which has been sterile filtered through 0.22 micron filter.

19. The formulation of any of claims 1-18, in lyophilized form.

20. The formulation of claim 19, which has been sterile filtered through 0.22 micron filter prior to lyophilization.

21. The lyophilized formulation of claim 19 or 20, which is stable at least for 9 months.

22. The formulation of any of claims 1-18 formulated as a solution or suspension in an aqueous system.

23. The formulation of claim 22, wherein the aqueous system is water.

24. The formulation of claim 22 or 23, which exhibits change in mean liposomal particle size of less than about 5% over a 3-day time course post reconstitution and dilution.

25. The formulation of any of claims 22-24 in injectable form.

26. The formulation of any of claims 1-25, which remains substantially free of crystalline antineoplastic drug for at least about 3 days.

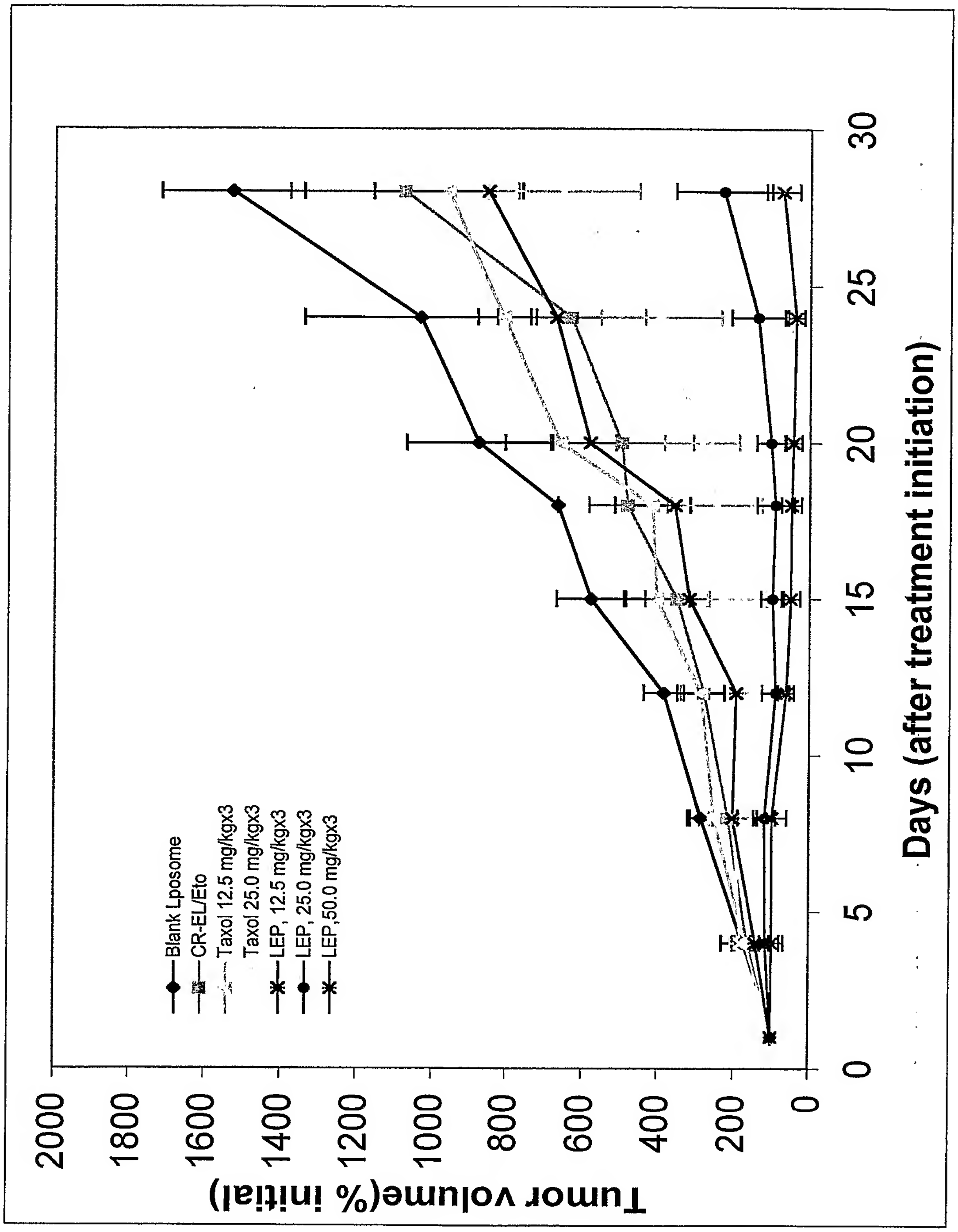


Figure 1

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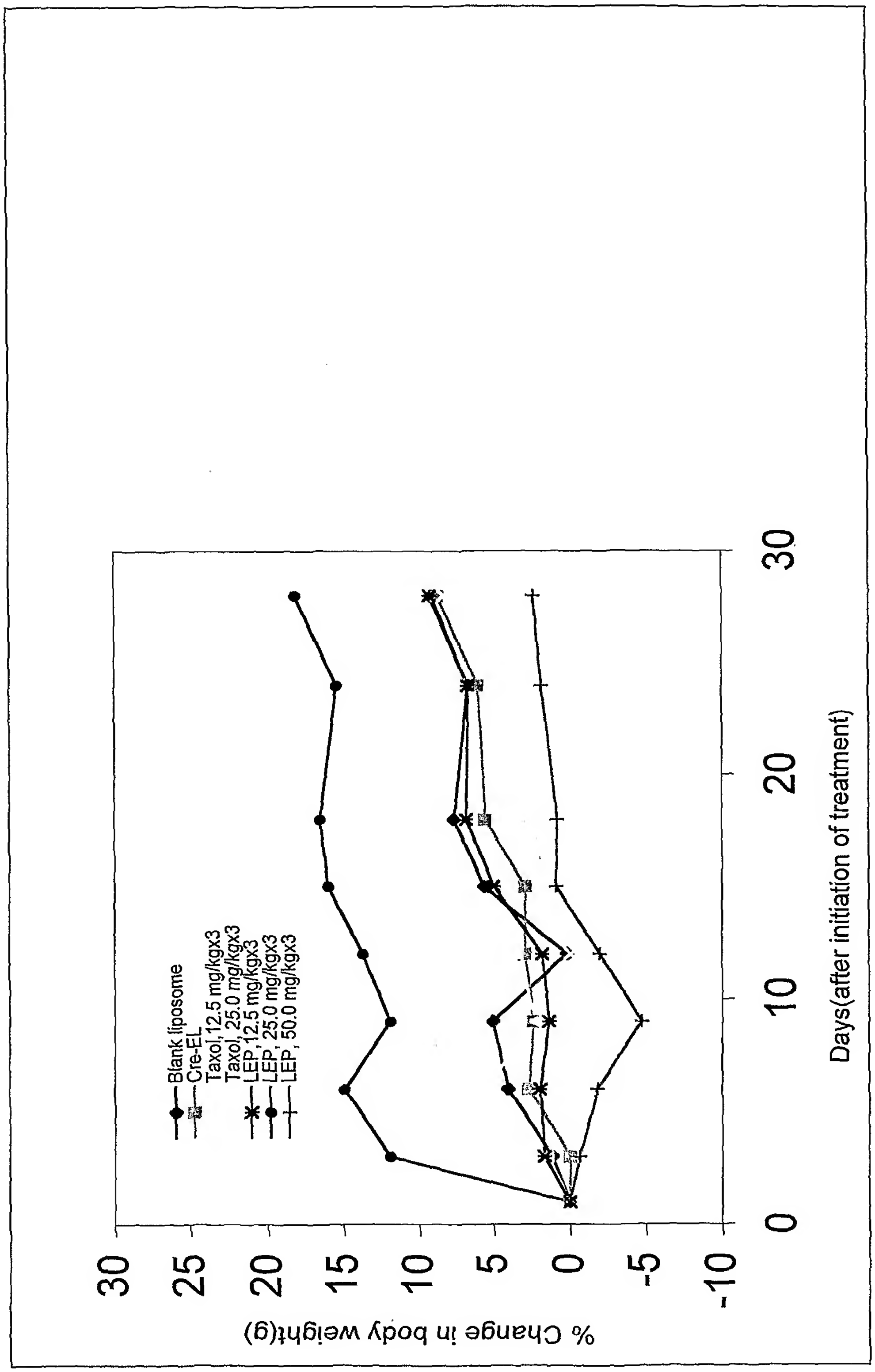


**Figure 2**

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(71) Applicant (*for all designated States except US*): **NEOPHARM, INC.** [US/US]; 150 Field Drive, Suite 195, Lake Forest, IL 60045 (US).

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(75) Inventors/Applicants (*for US only*): **ZHANG, Jia-Ai** [US/US]; 1251 Maidstone, Vernon Hills, IL 60061 (US). **UGWU, Sydney** [NG/US]; 4005 Stoney Island, Gurnee, IL 60031 (US). **MA, Lan** [CN/US]; 7606 Cascade Way, Gurnee, IL 60031 (US). **ANYARAMBHATLA, Gopal** [IN/US]; 1014 Lakehurst Dr., Apt. 104, Waukengan, IL 60083 (US). **AHMAD, Imran** [US/US]; 4731 Pebble Beach Drive, Wadsworth, IL 60083 (US).

(74) Agents: **HEFNER, M., Daniel** et al.; Leydig, Voit & Mayer, Ltd., Two Prudential Plaza, Suite 4900, 180 N. Stetson Avenue, Chicago, IL 60601-6780 (US).

(81) Designated States (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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(54) **Title:** STABLE STERILE FILTERABLE LIPOSOMAL ENCAPSULATED TAXANE AND OTHER ANTINEOPLASTIC DRUGS

(57) **Abstract:** The invention provides a formulation of one or more antineoplastic drugs encapsulated in liposomes including at least a lipid fraction in addition to the antineoplastic drug, wherein the composition is stable in an aqueous solution at room temperature.

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# INTERNATIONAL SEARCH REPORT

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**A. CLASSIFICATION OF SUBJECT MATTER**
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**B. FIELDS SEARCHED**

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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

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**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

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(71) Applicant (*for all designated States except US*): **NEOPHARM, INC.** [US/US]; 150 Field Drive, Suite 195, Lake Forest, IL 60045 (US).

(72) Inventors; and
(75) Inventors/Applicants (*for US only*): **ZHANG, Jia-Ai** [US/US]; 1251 Maidstone, Vernon Hills, IL 60061 (US). **UGWU, Sydney** [NG/US]; 4005 Stoney Island, Gurnee, IL 60031 (US). **MA, Lan** [CN/US]; 7606 Cascade Way, Gurnee, IL 60031 (US). **ANYARAMBHATLA, Gopal** [IN/US]; 1014 Lakehurst Dr., Apt. 104, Waukengan, IL 60083 (US). **AHMAD, Imran** [US/US]; 4731 Pebble Beach Drive, Wadsworth, IL 60083 (US).

(74) Agents: **HEFNER, M., Daniel** et al.; Leydig, Voit & Mayer, Ltd., Two Prudential Plaza, Suite 4900, 180 N. Stetson Avenue, Chicago, IL 60601-6780 (US).

(81) Designated States (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

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**Published:**
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WO 2004/069224 A3

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**(57) Abstract:** The invention provides a formulation of one or more antineoplastic drugs encapsulated in liposomes including at least a lipid fraction in addition to the antineoplastic drug, wherein the composition is stable in an aqueous solution at room temperature.

# INTERNATIONAL SEARCH REPORT

International Application No
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**A. CLASSIFICATION OF SUBJECT MATTER**
IPC 7 A61K9/127 A61K31/337

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS, EMBASE

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

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[X] Further documents are listed in the continuation of box C.

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|---|---|
| 8 September 2004 | 16/09/2004 |

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| European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016 | Epskamp, S |

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